UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PROGESTERONE INTRAVAGINAL DEVICES ON ARTIFICIAL INSEMINATION, OESTROUS SYNCHRONIZATION, OVULATION AND PREGNANCY RATE IN KEDAH-KELANTAN CATTLE

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: **Assoc. Prof. Dr. Abd Wahid Haron, D.V.M., Ph.D.**

Faculty: **Veterinary Medicine**

The first experiment was conducted to evaluate the effect of various progestagen treatment methods in term of oestrous synchronization, ovulation and pregnancy rate using controlled internal drug-release (CIDR) device or progesterone-releasing intravaginal device (PRID) with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection on the day of CIDR/PRID removal. Twenty-four non-pregnant Kedah-Kelantan (KK) cows were randomly assigned to two groups: CIDR (n=12); PRID (n=12). Both group were given 12-day synchronization treatment and received an intramuscular injection of 25 mg $PGF_{2\alpha}$ on the day of device removal. The ovaries of all cows were examined by ultrasonography, using a 5 MHz probe, from one day after CIDR/PRID removal until ovulation. Blood samples were collected for determination of progesterone. The proportion of cows observed in oestrus was significantly higher in the CIDR group than PRID group (91.7% vs 58.3%, $P<0.01$). Pregnancy rate was also significantly higher in CIDR group than PRID group

(27.3% vs 14.2%, $P<0.05$). Results from experiment indicated that CIDR is more efficient than PRID.

The second experiment was to evaluate the effect of the length of progestagen treatment (12 day vs 7 day) on oestrous synchronization, ovulation and pregnancy rate using controlled internal drug-release device (CIDR) with $PGF_{2\alpha}$ injection at the end of CIDR treatment. Sixteen adult non-pregnant KK cows were divided into two groups: long-term treated group (n=8); Short-term treated group (n=8).

day of CIDR removal. All the cows were undergone transrectal ultrasonography, and blood samples were taken for hormone assay. Until 120 h after CIDR removal, the percentage of cows came into oestrus was significantly higher in long-term-treated group than short-term treated group (87.5%

The pregnancy rate was significantly higher in the short-term treated group than in long-term-treated group (40.0% vs 25.0%,

follicle in long-term treated group attained a larger maximum diameter than in short-term treated group ($P<0.05$). The mean time of ovulation after device removal was 108 h in the short-term treated group and 88 ± 5.06 h in the long-term treated group; the length of time required for ovulation between two treatment were significantly different ($P<0.05$). The result showed that the 7-d CIDR+ $PGF_{2\alpha}$ oestrous synchronization protocol rendered a better fertility in Kedah-Kelantan cows.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KAJIAN KESAN ALAT INTRAVAGINA (CIDR ATAU PRID) KEATAS PERMANIAN BERADAS, PENYAMAAN GALAK, OVULASI DAN KADAR KEBUNTINGAN PADA LEMBU JENIS KEDAH-KELANTAN

Oleh

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Pengerusi: **Prof Madya Dr. Abd Wahid Haron, D.V.M., PH.D.**

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Kajian pertama dijalankan untuk mengenalpasti kesan kaedah perlakuan progestagen dengan menggunakan dua jenis alat intravagina iaitu alat CIDR (Controlled internal drug-release device) dan PRID (Progesterone-releasing intravaginal device) bersama suntikan prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) pada hari CIDR/PRID dikeluarkan ke atas penyamaan galak, ovulasi dan kadar kebuntingan. Dua puluh empat ekor lembu jenis Kedah-Kelantan yang dikenalpasti tidak bunting dibahagikan secara rambang kepada dua kumpulan: CIDR(n=12); PRID (n=12). Kedua-dua kumpulan diberi perlakuan penyamaan galak 12 hari dan satu suntikan 25mg $PGF_{2\alpha}$ di bahagian otot pada hari alat tersebut dikeluarkan. Ovari semua lembu diperiksa ultrasonografi dengan menggunakan 5 MHz proba dari satu hari selepas CIDR/PRID dikeluarkan sampai berlakunya ovulasi. Sampel darah diambil untuk penentuan paras progesteron. Kumpulan CIDR menunjukkan kadar kedatangan estrus amat bererti yang lebih tinggi berbanding dengan kumpulan PRID (91.7% vs 58.3%, $P<0.01$). Kadar kebuntingan amat bererti yang lebih tinggi

juga telah diperolehi pada kumpulan CIDR berbanding kumpulan PRID (27.3% vs 14.2%, $P<0.05$). Keputusan ujikaji menunjukkan bahawa alat CIDR adalah lebih efisyen berbanding dengan PRID.

Kajian kedua dijalankan untuk menentukan kesan perlakuan jangkamasa alat intravagina CIDR (12 hari vs 7 hari) ke atas penyamaan galak, ovulasi dan kadar kebuntingan. Enam belas ekor lembu jenis Kedah-Kelantan yang dikenalpasti tidak bunting dibahagikan secara rambang kepada dua kumpulan: kumpulan 12 hari (n=8) dan kumpulan 7 hari n=8). Kedua-dua kumpulan di beri satu suntikan 25mg $PGF_{2\alpha}$ di bahagian otot pada hari alat CIDR dikeluarkan. Semua lembu telah diperiksa dengan alat ultrasound melalui rectum dan sample darah diambil untuk ujian hormon. Sehingga 120 jam selepas CIDR dikeluarkan, kumpulan 12 hari menunjukkan kadar kedatangan estrus amat bererti yang lebih tinggi berbanding dengan kumpulan diperolehi pada kumpulan 7 hari (87.5% vs 62.5%, $P<0.05$). Kadar kebuntingan amat bererti yang lebih tiggi telah diperolehi pada kumpulan 7 hari berbanding dengan 12 hari (40.0% vs 25.0%, $P<0.05$). Follikel ovulasi di kumpulan 12 hari mencapai diameter maksimum yang lebih besar berbanding dengan kumpulan 7 hari ($P<0.05$). Kadar masa ovulasi selepas CIDR dikeluarkan adalah 108 jam bagi kumpulan 7 hari dan 88 ± 5.06 jam bagi kumpulan 12 hari ($P<0.05$). Jangkamasa ovulasi bagi kedua-dua kumpulan menunjukkan perbezaan yang bererti ($P<0.05$). Keputusan ujikaji menunjukkan bahawa perlakuan 7 hari CIDR + $PGF_{2\alpha}$ adalah kaedah penyamaan galak yang lebih sesuai untuk mendapat kesuburan yang lebih tinggi pada lembu jenis Kedah-Kelantan.

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I certify that an Examination Committee met on 5th November 2002 to conduct the final examination of Khor Chun Siah on his Master of Science thesis entitled "Effects of Progesterone Intravaginal Devices on Artificial Insemination, Oestrous Synchronization, Ovulation and Pregnancy Rate in Kedah-Kelantan Cattle" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Masters of Science. The member of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Khor Chun Siah

Date: 19/12/2002

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LIST OF ABBREVIATIONS

AI	Artificial insemination
CIDR	Controlled Internal Drug Release Device
CL	Corpus Luteum
CV	Coefficients of Variation
E_2	Oestradiol
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
IU	International Unit (s)
KK	Kedah-Kelantan
LH	Luteinizing Hormone
MARDI	Malaysian Agricultural Research and Development Institute
mg	milligram
ng	nanogram
P_4	Progesterone
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PRID	Progesterone-releasing Intravaginal Device
QC	Quality Control
r^2	Regression
SEM	Standard Error of the Mean

CHAPTER 1

GENERAL INTRODUCTION

Kedah-Kelantan (KK) cattle is the predominant breed of cattle in Malaysia and represents the majority of Zebu cattle (*Bos indicus*) in Malaysia. To date, there is a paucity of information on the reproductive performance of this breed. *Bos indicus* cattle are tolerance to heat stress, highly resistance to ticks, of good fertility and able to thrive on a low plane of nutrition (Frisch and Vercoe, 1984). However, they usually have lower potential for meat and lower fertility than *Bos taurus* (Dobson and Kamonpatana, 1986; Frisch *et al.*, 1987; Lamothe-Zavaleta *et al.*, 1991).

To overcome the small size of KK breed, crossbreeding programmes with exotic bulls were initiated by Malaysian Agricultural Research and Development Institute (MARDI) and other institutions in the country, in an effort to upgrade this breed. Artificial insemination (AI) could play an important role in the crossbreeding programme with breeds like Charolais, Limousin and Simental (Dahlan *et al.*, 1985).

AI is the most important and valuable single technique devised for the genetic improvement of animals (Odde, 1990), because it enables the widespread use of outstanding sires with valuable genetic potential to any livestock operation. Besides that, it facilitates progeny testing under a wide range of environmental and management conditions, thereby further improving accuracy

of selection leading to improved performance of the national herds. It also reduces risk of spreading sexually transmitted diseases like brucellosis, vibriosis (Ax *et al.*, 2000).

Generally, the success of an AI breeding programme depends on adequate facility, good herd management, well-trained personnel and accuracy of oestrus detection (Dahlan *et al*, 1985; Larson *et al*, 1995). To minimize labour and management requirements in modern beef cattle breeding system, the oestrous cycle must be synchronized so that a large number of treated females come into oestrus at the preset time (Schmitt *et al.*, 1994; Van Cleeff *et al.*, 1996; Pursley *et al.*, 1997; Ryan *et al.*, 1999).

There are two basic methods of synchronization of oestrous cycle in farm animals. The first method involves long-term administration of a progestagen so that the corpus luteum (CL) regresses naturally during the period when progestagen was administered. With this approach, the exogenous progestagen continues to exert a negative feedback on the luteinizing hormone (LH) secretion after regression of the CL. On progestagen withdrawal, follicular growth, oestrus and ovulation occur within 2 to 8 days. The second method induces the premature regression of a cyclic CL. The two primary luteolytic agents used are prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and oestrogen.

A combination of both progestagen and luteolytic agent can also be use to give better oestrous synchronization in cow. In this approach, luteolytic agent is used to regress the CL and progestagen in the form of intravaginal devices are

used to mimic the action of progesterone and prevent oestrus until its withdrawal. A fixed time luteolytic agent injection and removal of the progestagen will stimulate oestrus to occur within a specific period of time.

The objectives of this study were:

1. to determine the efficacy of two oestrous synchronization methods (CIDR versus PRID) in KK cows.
2. to document the response of KK cows to the two oestrous synchronization treatments.
3. to evaluate the effect of the length of CIDR treatment on follicular dynamics, oestrous synchronization and pregnancy rate in KK cows.
4. to determine the pregnancy rate after oestrous synchronization and AI.

CHAPTER 2

LITERATURE REVIEW

2.1 Kedah-Kelantan Cattle

2.1.1 Introduction

Kedah-Kelantan (Plate 1) is the most common breed of beef cattle found in Malaysia. They are widely found in the states bordering Thailand. Thus, they were once called Kedah-Siam, Kedah-Thai and Thai-Kelantan cattle (Devendra, 1975). Cameons (1981) has classified these cattle as indigenous Zebu. They were once used as draught animals but now served as an important source of domestic beef in Malaysia. With improved management and nutrition, the live weight gain of KK had increased, the weight of adult KK averaged 240 kg and 180 kg, for male and female, respectively (Devendra *et al.*, 1973).

Plate 1. An adult female Kedah-Kelantan cow

2.1.2 Origin and Description of Breed

The origin of this breed is uncertain, but it is possible that they come from Thailand, especially from the southern part where similar cattle are found. This fact is further reflected in they being concentrated in the states of Kedah, Kelantan and Trengganu; thus, the derivation of its name from the first two states.

Initially however, it appears that this breed owes its ancestry to the humped Chinese yellow cattle of South Chinese Zebu cattle (Epstein, 1969). The migration route into China started from Western Asia eastward initially and then south-west and eastwards into Burma, Laos, Thailand and Vietnam (Payne, 1970) and probably into Malaysia.

Using the classification of Payne (1970), these cattle can be categorised under the small short-horned or lyre-horned Zebu group like the series of breed of Bhutan, Nepal, Sikkim and northern India. The breed is a result of crossbreeding between the shorthorn-type cattle originating in west Asia and Zebu from India, notably the Kangyam and Ongole. The influence of the Ongole is probably more distinct, as the feature of this breed is quite prominent particularly in large populations of Kedah-Kelantan cattle.

The breed has in general, the characteristics of beef cattle, but lacks the prominence and blocky conformation identifiable in such improved beef breeds as the Brahman or the Simental. However, this breed is relatively small and has a

compact body. Brown is the most predominant colour but dark brown, black and white colours are also found. The lack of distinctive beef characteristics is due to lack of selection pressure and their continued use as draught animals (Devendra *et al.*, 1973).

2.2 The Oestrous Cycle of Cows

The oestrous cycle is defined as the time between periods of oestrus. The average length of oestrous cycle for cow is 21 days. However, individual variation can be seen in cows and cycles ranging from 17 to 24 days are considered normal. The duration of oestrus is 12-18 hours whilst the time of ovulation is 10-12 hours after the end of oestrus period (Allrich, 1994; Bernard *et al.*, 1983), respectively.

2.2.1 Stages of The Cow's Oestrous Cycle

The stages of oestrous cycle are oestrus, metoestrus, dioestrus and proestrus. These stages occur in a cyclic and sequential manner, except for the period of anoestrus (absence of cycling) which occurs in seasonal breeders such as the ewe, doe, and mare in the temperate regions. In most species, anoestrus also occur during pregnancy and early postpartum period.

Oestrus is defined as the period of time when the female is sexually receptive to the male and thus, will stand for mating. Oestrus lasts for 12 to18 hours in cows. There is also considerable variation in the oestrous cycle among

individuals. Cows under hot environment have shorter period of oestrus (10 to 12 hours) compared to the average of 18-hour for cows under cool climates (Valle *et al.*, 1994).

There is a great variation amongst individual cows in the intensity of oestrus signs; the manifestation tend to be more marked in heifers than in cows. However, it is generally agreed that the most reliable criterion that a cow or a heifer is in oestrus is that she will stand to be mounted by another cow or a male (Williamson *et al.*, 1972; Esslemount and Bryant, 1974; Foote, 1975).

In general, the female becomes more restless, irritable, and excitable during oestrus (Britt *et al.*, 1986). In addition, increased vocalization and interest in the male will become apparent if the male is in the vicinity. Pelvic adjustment into a mating position may occur. Cows are unique in that they display rather strong homosexual tendencies, making oestrus detection comparatively easy even when bulls are not present. Cows in oestrus will solicit mounts and attempt to mount other cows (Unal *et al.*, 1986). Cows that are coming into oestrus will mount cows that are in oestrus. However, mounting activity becomes more frequent when two or more cows are in oestrus than when a single cow is in oestrus (Britt, 1987). Frequency of mounting is higher at night than during the day, possibly because it is closer to the onset of oestrus; more mounting activity will be seen during early morning as compared to late afternoon (Pennington *et al.*, 1985). The cervix is relaxed and the uterus becomes turgid. Clinical signs of oestrus are congested vulva and clear mucus discharge from the vulva.

The period of metoestrus begins with the cessation of oestrus and lasts for about 3 days. Primarily, it is the period of corpus luteum formation. There is reduction in the amount of mucus secretion from the uterine, cervical and vaginal glands. However, ovulation occurs during this period in cows. Also, a phenomenon known as metoestrous bleeding occurs in some cows. During late proestrus and oestrus, high estrogen concentrations increase the vascularity of the endometrium. This vascularity reaches its peak about 1 day after end of oestrus. With declining estrogen level, some breakage of capillaries occurred, resulting in a small loss of blood (Sato *et al.*, 1982) and thus, metoestrous bleeding.

Dioestrus is characterized as the period in the cycle when the corpus luteum is fully functional. In the cow it starts about day 5 of the cycle, when an increase in blood concentration of progesterone can be detected, and ends with regression of the corpus luteum on day 16 or 17. The uterine glands undergo hyperplasia and hypertrophy, the cervix becomes constricted, the vaginal mucosa becomes pale and the secretions of the genital tract are scant and sticky. It has been called the period of uterine preparation for pregnancy (Geary and Reeves, 1992).

Proestrus is characterized as the period in the cycle when the corpus luteum regresses and extends to the start of oestrus. The principal distinguishing feature of proestrus is the occurrence of rapid follicle growth. Late during this period the effect of estrogen on the duct system and behavioral symptoms of approaching estrus can be observed. The uterus enlarges; the endometrium